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New Syntheses of α-Methyl- and α,α'-Dimethylspermine

N. A. Grigorenko*, J. Vepsalainen, A. Jarvinen***, T. A. Keinanen***, L. Alhonen***, J. Janne***, and A. R. Khomutov*[1]**

** Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, ul.Vavilova 32, Moscow, 119991 Russia*
*** Department of Chemistry, University of Kuopio, P. O. Box 1627 Kuopio, FIN-70211 Finland*
**** Virtanen Institute for Molecular Sciences, University of Kuopio, P. O. Box 1627, Kuopio, FIN-70211 Finland*

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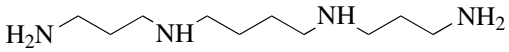
Abstract—α-Methylspermine and α,α'-dimethylspermine were synthesized in high overall yields starting from *N*-(benzyloxycarbonyl)-3-aminobutanol in order to study polyamine biochemistry *in vitro* and *in vivo.*

Key words: α,α'-dimethylspermine, α-methylspermine, polyamines, spermine/spermidine N^1-acetyltransferase

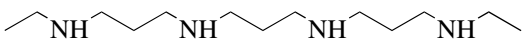
INTRODUCTION

Significant amounts of spermidine and spermine are present in all cell types and are essential for their normal growth.[2] Alkyl derivatives of Spd and Spm are widely used to control the enzymes of polyamine metabolism and to study the functions of Spm and Spd. Two groups of the analogues differing in the position of alkyl substituents are known. The first and the most studied group includes derivatives of Spm and its homologues that are symmetrically (e.g., DENSpm) or asymmetrically bisalkylated at terminal amino groups.

These compounds are actively transported into cells like Spm and Spd; however, they cannot fulfill the vital cellular functions of polyamines. The accumulation of DENSpm or similar compounds results in the induction of SSAT biosynthesis, which catalyses the key step of polyamine catabolism. The subsequent oxidation of N^1-Ac-Spm to Spd and N^1-Ac-Spd to Put results in the depletion of Spm/Spd pool and inhibition of cell growth (see reviews [1, 2]).



Spm



DENSpm

The analogues with alkyl substituents at carbon atoms of polyamine backbone constitute the second group of polyamine derivatives [3–5]; this is less investigated. In contrast to *N*-alkyl derivatives of Spm and like Spm and Spd, some of these compounds can support the growth of cells with depleted polyamine pool [3, 4]. It was shown [3] that α-methylpolyamines are not the substrates of SSAT, which provides their metabolic stability. At the same time, like Spm and Spd, these compounds can induce the biosynthesis of SSAT [6]. α-MeSpd, α-MeSpm, and α,α'-Me_2Spm interact with DNA as the natural polyamines [7]. In addition, α-MeSpd is the substrate of deoxyhypusine synthase, which executes a posttranslational modification of initiation factor eIF-5A necessary for the normal functioning of ribosome [8]. We have recently reported that α-MeSpd can prevent the development of acute pancreatitis caused by the depletion of polyamine pool resulting from the SSAT overinduction in SSAT-transgenic rats [9]. A small amount of Spm is known to significantly reduce the effective concentration of Spd necessary to support the growth of cells with polyamine deficiency [10]. It is probable that the same regularity would also be true in the case of α-methylated derivatives of Spm and Spd. Therefore, the use of catabolically stable Spm analogues (e.g., α-MeSpm and α,α'-Me_2Spm) that can fulfill crucial cellular functions of Spm should decrease the effective concentration of α-MeSpd.

[1] Corresponding author; phone: +7 (095) 135-6065; fax: +7 (095) 135-1405; e-mail: alexkhom@genome.eimb.relarn.ru

[2] Abbreviations: DENSpm, diethyl-*nor*-spermine; α-MeSpd, α-methylspermidine; α-MeSpm, α-methylspermine; α,α'-Me_2-Spm, α,α'-dimethylspermine; NS, *o*-nitrobenzenesulfonyl; PAO, polyamine oxidase; Put, putrescine (1,4-diaminobutane); SMO, spermine oxidase; Spd, spermidine (1,8-diamino-5-azaoctane); Spm, spermine (1,12-diamino-4,9-diazadodecane); and SSAT, spermine/spermidine N^1-acetyltransferase (EC 2.3.1.57).

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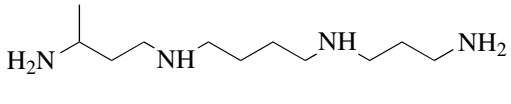
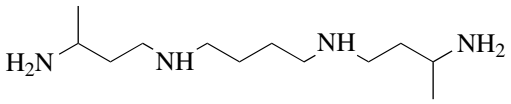
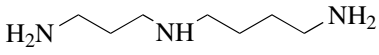


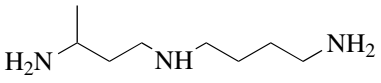
αMeSpm



α,α'-Me_2Spm



Spd



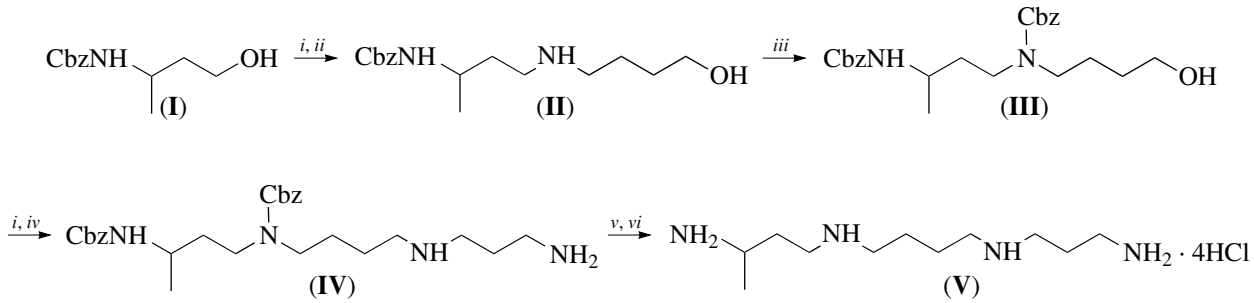
αMeSpd

The α-methylated analogues of Spm are still quite inaccessible despite their biochemical significance. We have recently described a simple synthesis of α-MeSpd [11]. In this paper, we present convenient schemes for the preparation of α-MeSpm and α,α'-Me_2Spm in amounts sufficient for studying their metabolism and biological effects *in vitro* and *in vivo*.

RESULTS AND DISCUSSION

Significant drawbacks are peculiar to the known preparation methods of α-Me-Spm and α,α'-Me_2-Spm; in particular, they provide the target compounds in dozen milligram scale with low overall yields [3].

Here we describe the building of α-MeSpm backbone by a successive elongation of polyamine backbone using the alkylation of amino components with mesylates of the corresponding *N*-protected amino alcohols (Scheme 1). The starting *N*-benzyloxycarbonyl-3-aminobutanol was prepared by the reduction of commercially available ethyl 3-aminobutyrate with $LiAlH_4$ in THF followed by the protection of amino group, using the method previously described for the synthesis of α-MeSpd [11].

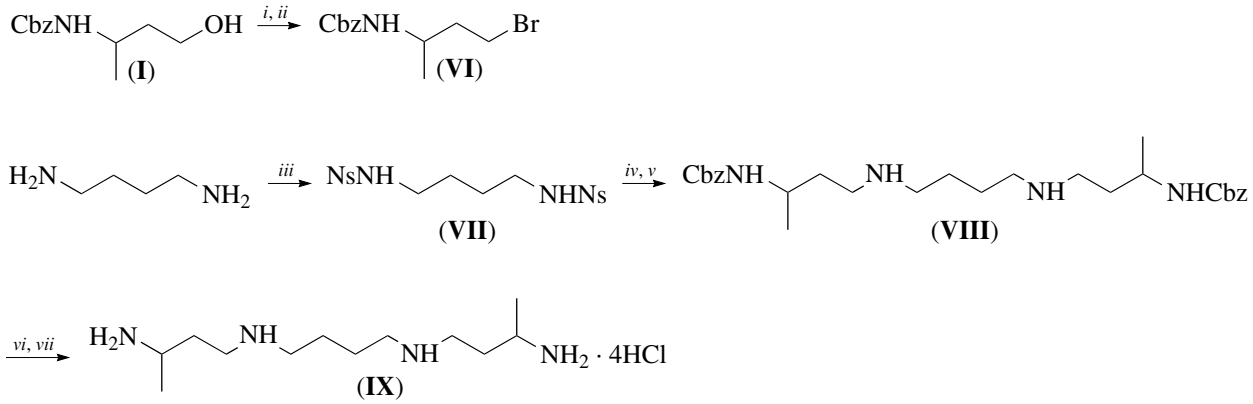


i, Ms-Cl/Et_3N/CH_2Cl_2; *ii*, $H_2N(CH_2)_4OH$/THF; *iii*, Cbz-Cl/H_2O/$NaHCO_3$; *iv*, $H_2N(CH_2)_3NH_2$/THF; *v*, H_2/Pd; *vi*, HCl/MeOH.

Scheme 1.

N-Cbz-Alcohol (**I**) was acylated with methanesulfonyl chloride at the first stage of the synthesis. The resulting mesylate was treated with excess 4-aminobutanol at 50°C without an additional purification. Crude amino alcohol (**II**) was purified by column chromatography on silica gel, its secondary amino group was benzyloxycarbonylated, and the completely *N*-protected amino alcohol (**III**) was treated with methanesulfonyl chloride. The reaction of mesylate of bis-Cbz-amino alcohol (**III**) with 10-fold excess of 1,3-diaminopropane in THF at +4°C [at 20°C, the number of minor byproducts increased, which made the purification of (**IV**) more laborious] led to bis-*N*-Cbz-protected α-methyl-Spm (**IV**). It was purified by column chromatography on silica gel. The catalytic hydrogenation of (**IV**) at atmospheric pressure over Pd black led to the removal of Cbz groups, and the target α-MeSpm tetrahydrochloride (**V**) was obtained in 41% total yield from *N*-Cbz-3-aminobutanol.

The above linear strategy was ineffective for the synthesis of α,α'-Me_2Spm. The symmetry of the target molecule allowed us to develop a convergent synthetic scheme (Scheme 2). Its key step was the alkylation of *N*,*N*'-bis-*o*-nitrophenylsulfonamide derivative of Put (**VII**) with *N*-Cbz-3-aminobutyl bromide (**VI**).



i, Ms-Cl/Et_3N/CH_2Cl_2; *ii*, LiBr/THF; *iii*, Ns-Cl/Et_3N/CH_2Cl_2; *iv*, (**VI**)/DMF/K_2CO_3; *v*, PhSH/DMF/K_2CO_3; *vi*, H_2/Pd; *vii*, HCl/MeOH.

Scheme 2.

The treatment of Put with 2.1 equiv of NsCl in the presence of Et_3N resulted in bis-sulfonamide (**VII**), which was difficultly soluble in most of organic solvents except for DMF and DMSO. Bromide (**VI**) was prepared by the successive conversion of *N*-Cbz-aminoalcohol (**I**) into mesylate and, then, without an additional purification, into the bromide by the treatment with excess LiBr in THF. After the separation of inorganic salts, (**VI**) was reacted with sulfonamide (**VII**) in the presence of K_2CO_3, which enabled the alkylation of both primary amino groups in a high yield. The attempts to use mesylate or tosylate of (**I**) for the alkylation of sulfonamide (**VIII**) led only to low yields of the target product. The selective removal of Ns groups with the retention of Cbz groups was achieved by the treatment with a small excess of thiophenol. The resulting (**VIII**) was easily purified by column chromatography on silica gel. At the final stage, Cbz groups were removed by hydrogenation over Pd black at atmospheric pressure and α,α'-Me_2Spm tetrahydrochloride (**IX**) was isolated with 57% total yield from *N*-Cbz-3-aminobutanol.

Thus, the synthetic schemes described in this paper help prepare α-MeSpm and α,α'-Me_2Spm in high total yields. The purity of the products was more than 99% as determined by HPLC under the standard conditions of polyamine analysis [12]. Our synthetic schemes are also suitable for the preparation of unknown (*R*)- and (*S*)-isomers of α-MeSpm and α,α'-Me_2Spm, which may exhibit different biological activities.

EXPERIMENTAL

Ethyl β-aminobutyrate, 1,4-diaminobutane, and *o*-nitrobenzenesulfonyl chloride were from Aldrich (United States); benzyl chloroformate, methanesulfonyl chloride, 4-aminobutanol, and 1,3-diaminopropane from Fluka (Switzerland). *N*-Benzyloxycarbonyl-3-aminobutanol was prepared according to procedure in [11].

TLC was carried out on precoated Kieselgel 60 F_{254} plates (Merck, Germany) using the following systems: (A) chloroform, (B) 200 : 1 chloroform–methanol, (C) 97 : 3 chloroform–methanol, (D) 9 : 1 chloroform–methanol, (E) 97 : 3 dioxane–25% ammonia, (F) 9 : 1 dioxane–25% ammonia, and (G) 4 : 2 : 1 : 2 *n*-butanol–acetic acid–pyridine–water. Kieselgel (40–63 μm, Merck, Germany) was used for column chromatography. The TLC spots of compounds were visualized in UV-light and using color reaction with ninhydrin.

^1H and ^{133}C NMR spectra were measured on a Bruker Avance 500 DRX spectrometer (Germany) using TMS ($CDCl_3$) and sodium 3-(trimethylsilyl)-propanesulfonate (D_2O) as internal standards. Chemical shifts are given in ppm, and constants of spin–spin coupling in Hz.

8-(Benzyloxycarbonyl)amino-5-azanonan-1-ol (II). A solution of methanesulfonyl chloride (1.24 ml, 16 mmol) in dry dichloromethane (10 ml) was added for 15 min to a stirred and cooled (0°C) solution of (**I**) (3.34 g, 15 mmol) and triethylamine (2.61 ml, 19 mmol) in dry dichloromethane (40 ml). Stirring was continued for 1 h at 0°C and for 1 h at room temperature. The reaction mixture was poured into 1 M NaHCO3 (25 ml); the organic layer was separated, successively washed with water (10 ml), 0.5 M H_2SO_4 (3 × 25 ml), water (10 ml), 1 M $NaHCO_3$ (20 ml), and saturated solution of NaCl (10 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in THF (20 ml) and 4-aminobutanol (8.9 g, 100 mmol) in THF (10 ml) was added in one portion. The reaction mixture was kept overnight at 50°C, THF and 4-ami-

nobutanol excess were then distilled off in a vacuum. The residue was distributed between 1 M NaOH (20 ml) and chloroform (25 ml); organic layer was separated, washed with saturated solution of NaCl (3 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in system E and the resulting solution was separated into three portions. Every portion was purified on a silica gel column (135 g) eluted with system E. The target fractions were evaporated and dried in a vacuum over phosphorus pentoxide to get (**II**); a viscous oil; yield 3.22 g [73% from (**I**)]; R_f 0.45 (F); ^{1}H NMR ($CDCl_3$): 7.40–7.29 (5 H, m, C_6H_5), 5.09 (2 H, s, $C\underline{H}_2Ph$), 5.03 (0.5 H, s, $N\underline{H}Cbz$), 5.01 (0.5 H, s, $N\underline{H}Cbz$), 3.85–3.77 (1 H, m, $CH_3C\underline{H}$), 3.57 (2 H, t, *J* 6.32, $C\underline{H}_2OH$), 2.70–2.55 (4 H, m, $C\underline{H}_2NHC\underline{H}_2$), 1.76–1.51 (8 H, m, NH + OH + $C\underline{H}_2CH(CH_3)NHCbz$ + $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.18 (3 H, d, *J* 6.54, CH_3).

***N*^{5},*N*^{8}-Bis-(benzyloxycarbonyl)-8-amino-5-azanonanol (III).** Benzyl chloroformate (1.55 ml, 11 mmol) was added in three portions with 15-min intervals to a cooled (0°C) and vigorously stirred mixture of (**II**) (2.94 g, 10 mmol), THF (10 ml), water (5 ml), NaHCO3 (0.84 g, 10 mmol), and 10 M NaOH (0.6 ml, 6 mmol). The stirring was continued for 1 h at 0°C and then for 3 h at room temperature. The aqueous layer was separated, extracted with chloroform (15 ml), and the combined organic phase was evaporated to dryness in a vacuum. The residue was dissolved in chloroform (30 ml); the solution was washed with 0.5 M H_2SO_4 (2 × 10 ml), water (15 ml), and 1 M $NaHCO_3$ (2 × 10 ml); dried with $MgSO_4$, and evaporated in a vacuum. The residue was dissolved in chloroform, and the resulting solution was separated into two portions. Every portion was purified on a silica gel column (120 g) eluted first with a 200 : 1 chloroform–methanol and then with a 95 : 5 chloroform–methanol mixtures to get (**III**); a colorless viscous oil; yield 3.94 g (92%); R_f 0.27 (C); ^{1}H NMR ($CDCl_3$): 7.34–7.27 (10 H, m, C_6H_5), 5.11 (2 H, s, $C\underline{H}_2Ph$), 5.07 (2 H, s, $C\underline{H}_2Ph$), 4.96 (0.5 H, s, $N\underline{H}Cbz$), 4.57 (0.5 H, s, $N\underline{H}Cbz$), 3.73–3.55 (3 H, m, $CH_3C\underline{H}$ + $C\underline{H}_2OH$), 3.30–3.18 (4 H, m, $C\underline{H}_2N(Cbz)C\underline{H}_2$), 1.75–1.45 (7 H, m, OH + $C\underline{H}_2CH(CH_3)NHCbz$ + $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.17–1.10 (3 H, m, CH_3); ^{13}C NMR ($CDCl_3$): 156.16, 155.90, 136.82, 128.52, 128.07, 128.00, 127.85, 67.07, 66.59, 62.33, 47.44, 45.41, 44.78, 44.34, 36.22, 35.13, 29.66, 25.02, 24.70, and 21.32.

***N*^{9},*N*^{12}-Bis-(benzyloxycarbonyl)-1,12-diamino-4,9-diazatridecane (IV).** A solution of MsCl (0.77 ml, 10 mmol) in dry dichloromethane (5 ml) was added for 10 min to a stirred and cooled (0°C) solution of (**III**) (3.85 g, 9 mmol) and Et_3N (1.53 ml, 11 mmol) in dry dichloromethane (20 ml). The stirring was continued for 1 h at 0°C and for 30 min at room temperature. The reaction mixture was poured into 1 M $NaHCO_3$ (20 ml); the organic layer was separated, washed with water (10 ml), 0.5 M H_2SO_4 (3 × 15 ml), water (10 ml), 1 M $NaHCO_3$ (10 ml), and saturated solution of NaCl (10 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in THF (15 ml), cooled to 0°C, and 1,3-diaminopropane (6.66 g, 90 mmol) in THF (5 ml) was added in one portion. The reaction mixture was kept for 6 h at 0°C, and overnight at room temperature. THF and 1,3-diaminopropane excess were then distilled off in a vacuum. The residue was dissolved in 1 M NaOH (15 ml); extracted with dichloromethane (15 ml); and the organic layer was separated and evaporated in a vacuum. The residue was dissolved in 94 : 6 dioxane–25% ammonia mixture, and the resulting solution was separated into two portions. Each portion was purified on a silica gel column (135 g) eluted with 94 : 6 dioxane–25% ammonia mixture. The target fractions were evaporated in a vacuum to dryness, and the residue was dried in a vacuum over phosphorus pentoxide to get (**IV**); a viscous oil; yield 3.05 g (70%); R_f 0.25 (E); ^{1}H NMR ($CDCl_3$): 7.34–7.29 (10 H, m, C_6H_5), 5.10 (2 H, s, $C\underline{H}_2Ph$), 5.07 (2 H, s, $C\underline{H}_2Ph$), 4.79 (1 H, s, $N\underline{H}Cbz$), 3.35–3.20 (5 H, m, $CH_3C\underline{H}$ + $C\underline{H}_2N(Cbz)C\underline{H}_2$), 2.73 (2 H, t, *J* 6.75, $C\underline{H}_2NH_2$), 2.66–2.53 (4 H, m, $C\underline{H}_2NHC\underline{H}_2$), 1.70–1.35 (11 H, m, NH + NH_2 + $C\underline{H}_2CH_2NH$ + $CbzNHCHC\underline{H}_2$ + $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.16–1.10 (3 H, m, CH_3); ^{13}C NMR ($CDCl_3$): 155.85, 136.88, 136.70, 128.48, 128.02, 127.92, 127.79, 67.07, 66.96, 66.48, 49.63, 47.85, 47.62, 47.17, 45.37, 44.77, 44.32, 40.54, 36.20, 35.06, 33.77, 27.25, 26.48, 25.95, and 21.33.

1,12-Diamino-4,9-diazatridecane tetrahydrochloride (α-MeSpm) (V). A suspension of Pd black in methanol (~0.9 ml) was added to a solution of (**IV**) (3.0 g, 6.2 mmol) in a 1 : 1 AcOH–MeOH mixture (30 ml), and hydrogenation was carried out at atmospheric pressure. The catalyst was filtered off, washed with methanol (20 ml), and the combined filtrates were evaporated to dryness in a vacuum. The residue was dissolved in methanol, diluted with 5 M HCl (7.4 ml), and the resulting solution was evaporated to dryness in a vacuum. The residue was recrystallized from a water–MeOH–EtOH mixture and dried in a vacuum over P_2O_5/KOH to give (**V**) tetrahydrochloride as colorless crystals; yield 1.97 g (88%); mp 250–251°C (dec.) {lit. [3]: mp 247°C (dec.) for lyophilized powder}; R_f 0.13 (G); ^{1}H NMR (D_2O): 3.51 (1 H, q, *J* 6.83, $CH_3C\underline{H}$), 3.24–3.11 (10 H, m, $C\underline{H}_2NH$ + $C\underline{H}_2NH_2$), 2.18–2.08 (3 H, m, $NHCHC\underline{H}_2$ + $C\underline{H}_2CH_2NH_2$), 2.04–1.96 (1 H, m, $NHCHC\underline{H}_2$), 1.81 (4 H, m, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.36 (3 H, d, *J* 6.60, CH_3); ^{13}C NMR ($CDCl_3$): 47.07, 45.41, 44.62, 44.00, 36.67, 30.47, 23.79, 22.83, 17.58, and 17.34. Found, %: C 36.03, H 9.00, N 15.04. $C_{11}H_{32}N_4Cl_4 \cdot 0.25H_2O$. Calculated, %: C 36.03, H 8.93, N 15.28.

***N*³-(Benzyloxycarbonyl)-amino-1-bromobutane (VI).** A solution of MsCl (1.16 ml, 15 mmol) in dry dichloromethane (10 ml) was added for 15 min to a stirred and cooled (0°C) solution of (**I**) (3.01 g, 13.5 mmol) and Et_3N (2.52 ml, 18.1 mmol) in dry dichloromethane (30 ml). Stirring was continued for 30 min at 0°C and for 30 min at room temperature. The reaction mixture was poured into 1 M $NaHCO_3$ (30 ml); the organic layer was separated, washed with water (10 ml), 0.5 M H_2SO_4 (3 × 15 ml), water (10 ml), and 1 M $NaHCO_3$ (10 ml); dried with $MgSO_4$; and evaporated in a vacuum. The residue was dissolved in THF (5 ml), and LiBr (3.5 g, 40 mmol) in THF (20 ml) was added in one portion. The reaction mixture was stirred overnight at room temperature and precipitated with chloroform (30 ml). The precipitate was filtered off, and the filtrate was evaporated in a vacuum. The residue was treated with chloroform (50 ml), washed with water (2 × 30 ml), 1 M $NaHCO_3$ (2 × 15 ml), dried with $MgSO_4$, evaporated in a vacuum and, then, dried in a vacuum over P_2O_5 /KOH to give (**VI**); a viscous oil; yield 3.78 g (95%); R_f 0.61 (A); ^{1}H NMR ($CDCl_3$): 7.37–7.26 (5 H, m, $C_6\underline{H}_5$), 7.11 (1 H, bd, N<u>H</u>Cbz), 5.03 (2 H, s, C$\underline{H}_2$Ph), 3.78–3.72 (1 H, m, $CH_3C\underline{H}$), 3.46–3.41 (2 H, t, *J* 7.38, C$\underline{H}_2$Br), 2.10–1.99 (1 H, m, $CH_3CHC\underline{H}_2$), 1.95–1.85 (1 H, m, $CH_3CHC\underline{H}_2$), and 1.12 (3 H, d, *J* 6.63, CH_3). It was used without further purification.

Bis-*N*¹,*N*⁴-*o*-nitrobenzenesulfonyl-1,4-diaminobutane (VII). A solution of *o*-nitrobenzenesulfonyl chloride (6.05 g, 27.3 mmol) in dry dichloromethane (30 ml) was added for 40 min to a cooled (0°C) and vigorously stirred solution of freshly distilled 1,4-diaminobutane (1.1 g, 12.5 mmol) and Et_3N (5.2 ml, 37.5 mmol) in dry dichloromethane (50 ml). Stirring was continued for 1 h at 0°C and 3 h at room temperature. The precipitate was filtered off, washed with MeOH (3 × 15 ml), chloroform (3 × 10 ml), and dried over P_2O_5 to give (**VII**), yield 5.3 g (91%) as a pale-yellow crystals; R_f 0.55 (B); mp 185–186°C; ^{1}H NMR (DMSO-d_6): 7.98–7.91 (4 H, m, C_6H_4), 7.86–7.81 (4 H, m, C_6H_4), 2.87–2.82 (4 H, bt, C$\underline{H}_2$NHNs), 1.44-1.37 (4 H, m, $CH_2C\underline{H}_2C\underline{H}_2CH_2$).

Bis-*N*²,*N*¹³-(benzyloxycarbonyl)-2,13-diamino-5,10-diazatetradecane (VIII). A suspension of bromide (**VI**) (3.78 g, 13.2 mmol), bissulfonamide (**VII**) (2.16 g, 4.7 mmol), and K_2CO_3 (4.30 g, 31 mmol) in anhydrous DMF (30 ml) was stirred for 48 h at room temperature. DMF (10 ml), K_2CO_3 (3.7 g, 27 mmol), and PhSH (1.4 ml, 13.5 mmol) were then added to the reaction mixture, and stirring was continued for 12 h at room temperature. The reaction mixture was evaporated to dryness in a vacuum. The residue was treated with chloroform (50 ml); the precipitate was filtered off and washed with chloroform (3 × 10 ml); and the combined filtrates were washed with water (2 × 30 ml). Chloroform was distilled off in a vacuum, and the residue was dissolved in 1,4-dioxane (10 ml) and purified on a silica gel column (130 g) eluted with system (E). After evaporation in a vacuum and drying the residue in a vacuum over phosphorus pentoxide, (**VIII**) was obtained as colorless crystals; yield 2.3 g [70% from (**VI**)]; R_f 0.46 (E); mp 107–108°C; ^{1}H NMR ($CDCl_3$): 7.36–7.26 (10 H, m, $C_6\underline{H}_5$); 5.51 (2 H, bs, N<u>H</u>Cbz), 5.08 (4 H, s, C$\underline{H}_2$Ph), 3.85–3.74 (2 H, m, $CH_3C\underline{H}$), 2.73–2.65 (2 H, m, C$\underline{H}_2$NH), 2.64–2.54 (2 H, m, C$\underline{H}_2$NH); 2.54–2.45 (4 H, m, C$\underline{H}_2$NH), 1.72–1.40 (8 H, m, C$\underline{H}_2$CHNHCbz + C$\underline{H}_2$CHNHCbz + $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.16 (6 H, d, *J* 6.48, CH_3); ^{13}C NMR ($CDCl_3$): 156.03, 136.86, 128.53, 128.05, 67.14, 66.41, 49.82, 46.45, 46.01, 36.66, 27.87, and 21.28.

2,13-Diamino-5,10-diazatetradecane tetrahydrochloride (α,α'-Me₂Spm) (IX). A suspension of Pd black in methanol (~0.8 ml) was added to a solution of (**IV**) (2.3 g, 4.6 mmol) in a 1 : 1 AcOH–MeOH mixture (30 ml), and hydrogenation was carried out at atmospheric pressure until the evolution of carbon dioxide ceased (for approximately 3 h). Pd black was filtered off, washed with MeOH (15 ml), and the combined filtrates were evaporated to dryness in a vacuum. The residue was dissolved in methanol (15 ml), diluted with 5 M HCl (6.0 ml), and the resulting solution was evaporated to dryness in a vacuum. The residue was recrystallized from a water–MeOH–EtOH mixture, and the resulting colorless crystals of (**IX**) were dried in a vacuum over P_2O_5/KOH; yield 1.47 g (85%), mp 224–225°C (dec.) {lit. [3]: mp 180°C (dec.) for a lyophilized powder}; R_f 0.13 (G); ^{1}H NMR (D_2O): 3.55–3.46 (2 H, m, $CH_3C\underline{H}$), 3.23–3.18 (4 H, t, *J* 6.77, C$\underline{H}_2$NH), 3.17–3.11 (4 H, m, C$\underline{H}_2$NH), 2.18–2.09 (2 H, m, C$\underline{H}_2$CHNH$_2$), 2.04–1.93 (2 H, m, C$\underline{H}_2$CHNH$_2$), 1.84–1.75 (4 H, m, $CH_2C\underline{H}_2C\underline{H}_2CH_2$), and 1.35 (6 H, d, *J* 6.63, CH_3); ^{13}C NMR (D_2O): 49.86, 48.14, 46.78, 33.27, 25.64, and 20.09. Found, %: C 38.08, H 9.24, N 14.67. $C_{12}H_{34}N_4Cl_4$. Calculated, %: C 38.31, H 9.11, N 14.89.

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